NUTRITION REVIEWS

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THE CANADIAN NUTRITION PROGRAM

The Nutrition Program sponsored by the Nutrition Services of the Canadian Department of Pensions and National Health consists of (a) an advisory and educational effort concerning the adequate feeding of war workers, (b) advice and assistance to the public directly and through provincial and local nutrition committees, together with coordination of such educational work by private and voluntary agencies, and (c) other advisory and research activities in nutrition.

The problem of improving the diet of war workers includes inspection of war industries and provision of canteens or cafeterias as well as the quality of the food served. Establishment of suitable food services can be required under penalty. A survey throughout Canadian war industries has included over 500 inspections, covering over 100 cafeterias, nearly 200 canteens, and a variety of other food facilities.

These inspections and other methods have afforded contact with about 500,000 war workers. Reinspections are being carried out to ensure proper operation. This advisory service includes assistance with material and equipment priorities, discussion of plans and blueprints, preparation of menus, and other features. During the past year this service has taken up most of the staff available. It is now being sought even ahead of actual inspections and the current year promises to increase the demands greatly. In addition to inspections and advice, assistance is given to war industries in informing their employees about nutrition and its importance in health. Pay check leaflets, booklets, posters, lectures, movies, and exhibits have been used, and more are being planned for this year. Not all of this is handled from the federal office since, in suitable cases, local community groups assist in the arrangements, and other governmental groups take part.

For the public the past year has seen a slow period of organization for establishing provincial nutrition committees, and for getting paid nutritionists attached to public health departments and to various welfare associations. One of the most serious problems right now is finding some way of training such nutritionists so that the work carried out may be as uniform as possible. This course seems best for a long term and all-year-round effort to spread nutritional education, but voluntary assistance is also necessary and has been extensively mobilized in Canada. Where two years ago there were only 7 communities in all Canada with a nutrition campaign in progress, there are now 80, and more are starting weekly. This work has been greatly helped and public interest has been aroused by generous support from advertisers, magazines, and newspapers during December, January, and February. Some of this commercial support is continuing.

The progress of nutritional education alone is rather slow and apt to be disappointing without other governmental assistance. Our goal in this field is that there may be a time when it will be difficult to be poorly fed. This goal is bound up with adequate agricultural production of the proper foods, with a high nutritional standard for storing and processing foods, with food allocations to certain groups on a nutritional basis, and with the economics of the consumer's ability to buy. Even with the best information the public does not always use increased purchasing power in the best manner (nutritionally), so that other assistance is needed. Some of these

problems are acute right now, when the supply of food is short, and some must await a postwar solution. There is clearly a need for nutritional advice in various boards, committees, and conferences, and "Nutrition Services" has made available such information on many occasions, both sought and unsought. This role is partly that of a liaison between such committees and advisory bodies as the Canadian Council on Nutrition.

In the field of research only a small beginning has been made. A compilation of all food analyses made in Canada has been almost completed. Studies on special phases of food composition are in progress. Surveys of dietary status of various groups of people have been made, and it is hoped that they may be extended to keep an account of the effects of war restrictions.

Thus on four fronts—war industries, general information, consultation, and research—the Canadian Nutrition Program is moving forward.

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CORNEAL VASCULARIZATION AS A SIGN OF ARIBOFLAVINOSIS

Early signs of vitamin deficiencies are important as indices for nutritional survey work and to aid in curtailment of the development of more severe pathology. If, however, the lesion is a minor one and occurs in organs such as the gums, tongue, or eyes which are sensitive to so many other conditions, it is inevitable that these signs are not always entirely specific for a vitamin deficiency.

In 1939 Bessey and Wolbach (*J. Exp. Med.* **69,** *1 (1939)*) described a corneal vascularization as the first morphologic manifestation of riboflavin deficiency in rats. This consists of an invasion of capillaries toward the center of the cornea. Edema and cellular infiltration result in corneal cloudiness. In more advanced stages, the superficial epithelium becomes separated from the deeper layers and presents a markedly changed appearance. In man, Kruse, Sydenstricker, Sebrell, and Cleckley (*Pub. Health Reports* **55,** *157 (1940)*) also described superficial vascularizing keratitis as an early and easily recognized sign of riboflavin deficiency. Subjectively, photophobia, dimness of vision, burning sensations in the eye, soreness and swelling of the eyelids, and impairment of visual acuity are common complaints. The earliest corneal lesion may be recognized by the slit-lamp; later changes are usually grossly visible.

There is no question that these lesions may occur during riboflavin deficiency, but recent reports make it probable that they also occur in various unrelated instances. The object of a report by Sandstead (*Pub. Health Reports* **57,** *1821 (1942)*) was to study the prevalence of superficial vascularization of the cornea in various population groups and the effect of riboflavin therapy on this condition. Three hundred and sixty-six persons were examined with a binocular corneal microscope with slit-lamp illumination. The extent of the lesion was estimated by the number of capillary loops and the extent of penetration toward the center of the cornea. Two examiners made independent observations. Eighty to 95 per cent of the persons examined showed some degree of corneal invasion by capillaries. This was more frequent and severe in older groups than in schoolchildren. To study the effect of riboflavin therapy, 17 subjects were given 5 mg. of riboflavin three times daily for sixty days; 12 control subjects received placebo capsules. The

division of subjects was made by a third person unaware of the therapy status. The corneal vascularization of those who had received riboflavin was not significantly different from that of persons in the control group. Likewise, in a group of 22 schoolchildren, similarly divided, no apparent therapeutic effect was observed.

Sandstead believes that various degrees of capillary invasion of the cornea may result from exanthematous diseases, particularly measles, where an acute catarrhal conjunctivitis is common. He concludes that "at the present time, therefore, it seems doubtful that superficial vascularization of the cornea, as observed in this study and as found in the general population, should be considered a sign of riboflavin deficiency; further controlled investigations along this line should be made."

Similar conclusions were reached in England by Scarborough (*Brit. Med. J.* **II,** *601* (*1942*)). In this study 204 patients in the outpatient department of the Edinburgh Royal Infirmary were examined for evidence of capillary invasion of the cornea. Examination was made according to the directions of Dr. Sydenstricker, but in no case was the slit-lamp used. The diagnosis of the typical lesion was in fact confirmed by Dr. Sydenstricker who was visiting England at the time. Seventy patients, or 34 per cent, showed evidence of corneal lesion and incidence was greatest in patients over 50 years of age (68 per cent). Eight cooperative subjects were given riboflavin, orally or parenterally, and with the possible exception of one subject, none showed any obvious difference in the degree of the corneal lesion. The period of therapy did not exceed one month.

In contrast to this group, 8 subjects who had frank evidence of vitamin deficiency disease and all of whom likewise showed the corneal lesion, were treated with riboflavin. Of these, 3 were cured and 2 definitely improved, with respect to the corneal lesion, by riboflavin within a period of two to four weeks. At least 3 of these patients showed additional signs of riboflavin deficiency such as cheilosis and glossitis.

Scarborough, therefore, believes that because of the high incidence of capillary invasion of the cornea in individuals showing no other signs of vitamin deficiency, and their negative response to riboflavin therapy, this lesion cannot by itself be considered conclusive evidence of ariboflavinosis.

Youmans and Patton (Clinics **1,** *303* (*1942*)) observed a number of subjects with corneal vascularization in whom there was no correlation with dietary riboflavin and no other physical evidence of riboflavin deficiency. Reexamination showed less corneal vascularization in a winter or spring period in spite of a lower intake of riboflavin. They conclude that corneal vascularization is not itself diagnostic of riboflavin deficiency.

According to Kruse's "Concept of the Deficiency State," a slowly developing chronic lesion may require a long period of treatment for its reversal. This concept was supported, or perhaps arrived at, in part, by the finding that certain ocular, gingival, and tongue lesions attributed to particular vitamin deficiencies were only partially cured by or did not respond at all to treatment with large amounts of the specific vitamins even after several months. At least in the case of riboflavin deficiency, Scarborough prefers to believe that the result of a therapeutic test is probably still the best criterion of whether a given lesion is characteristic of the vitamin deficiency. He has in his support those cases of corneal lesions more obviously associated with riboflavin deficiency which promptly responded to therapy. The discussion perhaps resolves into testing, under more rigorous experimental conditions, whether or not a lesion produced slowly with a mild deficiency state requires an extremely long period of therapy for reversal, and also whether or not a variety of other disease conditions, independent of the vitamin deficiency, result in indistinguishable lesions. Certainly today the vast majority of competent clinical investigators favor the latter view.

A SHORT METHOD FOR DIETARY ANALYSIS

The need for dietary evaluation in terms of specific nutrients and classes of nutrients has been frequent in the past but it will be even more necessary in the immediate future as a check upon the probable adequacy of food intake for the individual and for groups of individuals. As food rationing becomes more strict, studies of the approximate nutritive value of different dietaries will be of great practical value. A feasible approach to this problem is suggested in an article by Donelson and Leichsenring of the University of Minnesota (*J. Am. Dietet. Assn.* **18,** *429 (1942)*). These authors have devised a short method for dietary "analysis" based on the use of representative mean values for the composition of various groups of foods. These means are derived from nutritive values for the most commonly eaten foods as indicated by a study of typical dietaries in the University of Minnesota area. They are computed on "average size" servings. In obtaining the mean values for a food group, figures for certain foods are weighted in accordance with the frequency with which these foods occur in the dietary. For example, in arriving at the mean value for citrus fruits it was found that oranges were eaten twice as often as grapefruit. The values for oranges, therefore, were multiplied by two before the means were derived.

In determining the groups to which the various fruits and vegetables belong, it was necessary to take into consideration the differences in composition as well as the similarities. Owing to special differences, it was necessary to divide vegetables, for example, into six groups as follows: (1) cabbage, (2) green leafy vegetables, (3) green and yellow vegetables, (4) potatoes, (5) other vegetables, and (6) tomatoes. Separate groups were necessary for cabbage and tomatoes since these foods have a higher vitamin C content than the other vegetables in the groups in which they might otherwise be classified, and owing to the frequency with which they occur in the American diet a marked error might otherwise result. Furthermore, their dissimilarity in vitamin A content prevents grouping them together.

The data for 30 different food groups were collected in a single table. Both the composition and the approximate measure of average servings are given for each of the constituents comprising the group. The nutritive value of a diet can be ascertained by summarizing a day's menu as: refined cereals, 7 servings; whole grain cereals, 1 serving; butter, 4 servings; milk, 2½ servings; etc. The food values for the group times the number of servings quickly indicates the amount of the various essential nutritive factors.

The authors indicate, from statistical considerations, a high degree of accuracy for their short method. The time needed for computing a series of diets by this method is 40 per cent less than that required for usual dietary analyses.

This simplification should encourage the practice of checking diets for their nutritive values—for calories, protein, calcium, phosphorus, iron, vitamin A, thiamine, riboflavin, and ascorbic acid.

A short method of calculating the nutritive value of diets is also presented by Berryman and Chatfield (*J. Nutrition* **25,** *23 (1943)*). The underlying principles are similar to those used by Donelson and Leichsenring but the classification of 17 food groups follows the amounts of various factors per pound. Inasmuch as these data are intended for use in the nutritional accounting of mass feeding, especially of the Army and Navy, the classification of Howe, Pritchett, and Berryman (*J. Am. Dietet. Assn.* **18,** *435 (1942)*) is used. In selecting the figures for each item, care was taken to choose the grade, kind, and form of food appropriate to army use. The

authors state that while this short method is valuable for appraising the army ration, it may require adaptation to fit the food habits or pattern of other groups.

DIET AND LACTATION

Dietary factors are involved in the initiation and maintenance of lactation. Knowledge of this is important because failure of lactation is recognized as a major problem by obstetricians.

Until recently lactation has been thought to be largely the result of hormone stimulation and control. Recent findings reported from several nutrition laboratories indicate that this concept must be modified. Japanese workers were the first to postulate dietary lactation factors, L_1 and L_2, that were required for initiation and support of the lactating function in the rat. Sure (*J. Nutrition* **22,** *499* (*1941*)) presented evidence from rat studies, that p-aminobenzoic acid and inositol were lactation factors. This was confirmed by Climenko and McChesney (*Proc. Soc. Exp. Biol. Med.* **51,** *157* (*1942*)) and also by Ross, Phillips, and Bohstedt (*J. Animal Sci.* **1,** *353* (*1942*)) who used brood sows as well as rats.

Folley, Henry, and Kon (*Nature* **150,** *318* (*1942*)) reported the effect produced by a purified diet on reproduction and lactation of the rat. These authors used a diet composed of sugar 60, flavin-free casein 20, lard 3, ground nut oil 12, and salts 5 per cent, supplemented with thiamine, riboflavin, pyridoxine, niacin, inositol, choline, calcium pantothenate, p-aminobenzoic acid, 2-methyl-1,4-diacetyl naphthohydroquinone, cod liver oil, and alpha-tocopherol. Three successive generations of rats were born and reared on this diet. The lactation of the experimental animals was subnormal compared with control animals. Folley and co-workers failed to confirm the work of the Japanese. However, it is possible that some of the factors that were fed as crystalline vitamins were identical with the factors L_1 and L_2 postulated by the Japanese to be necessary to support normal lactation. Folley's diet included p-aminobenzoic acid and inositol. Factors L_1 and L_2 might be identical with these two vitamins. Extension of Folley's work, including omission and graduated restriction of individual factors from the diet, is awaited with interest.

The evidence at present indicates that dietary factors, possibly specific ones, are necessary for initiation and support of lactation, a finding which might come to be of great importance in its application to human beings.

FOOD FOR OVERSEAS FORCES

The problem of satisfying the dietary requirements of overseas forces has many ramifications involving such varied questions as food requirements for various types of troops, palatability, keeping quality, packaging, space required during transportation, facilities for mass production, and protection from contamination. These are the types of problems which the Quartermaster's Subsistence Research Laboratory has been studying with considerable success. This laboratory has the difficult assignment of finding ways and means of furnishing all necessary information regarding the above mentioned questions, in order that army rations may be palatable and nutritious under adverse conditions of shipment and storage.

Interesting phases relative to food requirements of overseas forces have been

covered admirably by Wodicka (*Ind. Eng. Chem.* **35,** *12 (1943)*). Where it is possible to do so, overseas troops are fed Field Ration A, which is composed of perishable and nonperishable foods purchased, in part, in the area in which the troops are stationed. Master menus are submitted to service commands by the Quartermaster General. Food purchases are based on these menus.

Where food supplies are restricted and perishable foods cannot be furnished from this country, the men are given Ration B which is composed of nonperishables such as sugar, flour, salt, and canned or dehydrated meats, fruits, and vegetables. These are the types of rations fed to garrison troops both here and overseas.

"Operational rations" are issued where field kitchens cannot be used. Ration C is the chief operational ration and consists of six cans of food. Three of these, one for each meal, are identical—containing hard candy, special biscuits, sugar, and soluble coffee. The other three cans, one for each meal, contain, respectively, meat and beans, meat and vegetable stew, and meat and vegetable hash. These can be eaten hot or cold. Wodicka states that while this ration is better than the old "iron rations" of World War I, it is now considered somewhat obsolescent.

Ration D is an emergency ration containing three 4 oz. chocolate bars that will not soften at high temperatures. These are reinforced with thiamine. The so-called "combat ration" is known as Field Ration K. Designed for parachute troops, this ration is likely to replace Ration C for combat troops. Three moisture and gas resistant packages, one for each meal, make up the day's requirement. Each package contains two types of biscuits, canned meat or cheese, a confection, a beverage concentrate, chewing gum, and four cigarettes. Canned meat, beverage concentrate, and confections are different for each meal. Wodicka states that tests in the field have shown that this ration is well suited for maintaining fighting efficiency and morale.

Wodicka states that the four guiding principles which must be used in designing a satisfactory ration are palatability, nutritional value, stability, and "caloric density." The latter refers to the maximum calories that can be concentrated into packages of a given size, to conserve shipping space. Instead of referring to "calories per ounce," subsistence officers now talk in terms of "calories per cubic inch." There are other factors which require study, such as ease of preparation, presumptive production capacity, cost, and physiologic effect.

Wodicka terminates his paper with an appeal for cooperation from nutrition specialists and food technologists in assisting the Quartermaster's Subsistence Research Staff in finding new and attractive food products which will meet the rigid specifications enumerated. He states that satisfactory desserts and stable sources of vitamin C are needed.

Another important phase of the overseas food problem is that of packaging. Melson (*Ind. Eng. Chem.* **35,** *16 (1943)*) describes the types of packages that are required to meet the needs of overseas forces. He points out that food containers must be able to withstand the rigors of arctic and tropical climates. Protection must be afforded against moisture vapor, gases, odor transfer, grease transfer, insects, and rats. Melson lists the various materials used in manufacturing food containers and gives reasons why certain types of materials must be specified for special types of foods. Conditions are specified for the use of such materials as tinned containers, bonderized plate, lacquered metal, fiber cans, enameled steel, and the various types of laminated paper containers.

An interesting description is given of the research that has been done to find satisfactory films of plasticizing agents, waxes, coatings, lacquers, and adhesives for the manufacture of moisture proof container

materials that will be suitable for dehydrated foods. Excellent progress is reported although few details are given. Most of these new products have come into being because of the lack of critical materials.

The efficiency of various types of containers in protecting foods from war gases is discussed by Katz of Edgewood Arsenal (*Ind. Eng. Chem.* **35,** *20* (*1943*)). He points out that chemical warfare agents in liquid or finely divided solid form are likely to be more harmful than those in the form of gases or vapors. Since decontamination is difficult, it is the objective of the army specialists to protect the food completely from contamination, by suitable wrappings and coverings.

While hermetically sealed containers of glass, metal, or glazed earthenware provide perfect protection, excellent results have been obtained with cellophane films, multiple wrappings of heavy, well sized paper, and certain types of waxed and coated papers. Glue and sodium silicate, properly applied, will make corregated paper cartons resistant to chemical warfare agents. Methods of salvaging contaminated foods are discussed. Emphasis is placed on the necessity of proper inspection by qualified medical and chemical warfare officers before decontaminated foods should be used.

It would appear that there is truth in the statement that the American soldier is the best fed soldier in the world. It is also a matter of pride to know that our military leaders are not satisfied with the excellent progress already made but are seeking cooperation from scientists and technologists, with the hope of furnishing our combat troops with rations which compare favorably in palatability and which may actually be of higher nutritional value than the diets to which they have been accustomed at home.

BIOTIN METABOLISM IN MAN

Biotin shares with vitamin K the distinction of being one of the vitamins known to be supplied to man in large amounts by intestinal bacteria—in some cases the amount from this source exceeding that supplied by the diet. In order to induce biotin deficiency in human beings, Sydenstricker and associates had to feed large amounts of egg white or "avidin" to bind the biotin in the intestinal tract and thus prevent its absorption (*Nutrition Reviews* **1,** *18* (*1942*)). Because of the practical difficulty of measuring vitamin K itself, no nutritional balance studies for vitamin K have been made. The observations by Oppel (*Am. J. Med. Sci.* **204,** *856* (*1942*)) on biotin metabolism in man therefore have a special interest; they are perhaps the first balance studies made on human beings of an essential nutritional substance which does not come primarily from food.

Oppel, using the technic described by du Vigneaud and associates (*J. Biol. Chem.* **140,** *643* (*1941*)), found that biotin is present in the urine of human beings in the free rather than in the combined form. Normal subjects eliminated between 14 and 111 micrograms per day; the great majority excreted between 20 and 50 micrograms per day. There was no essential difference between men and women in this regard. When a subject consumed an unrestricted diet, the daily urinary output varied often by more than 100 per cent, but when the same diet was consumed each day, the excretion was remarkably constant. While variations existed within the twenty-four hour period and excretion was higher during the day than at night, there was no sharp increase of biotin in the urine following meals. One subject, however, showed a distinct flooding of biotin in the urine during the hours immediately following a large test dose. The urinary output was

also studied on 60 patients with a wide variety of pathologic conditions. Practically all values fell within the normal range. In general, patients whose food intake had been conspicuously low excreted relatively small amounts of biotin, but there appeared to be a level at or near the lower range of normal values below which the excretion did not fall even during starvation.

In the second portion of his study, Oppel performed balance studies on a small group of patients. Each patient was given the same diet each day but several different types and combinations of diets were tried. The biotin content of the food varied, by actual determination, from 30 to 65 micrograms per day. The most significant increase in dietary biotin was effected when liver was included with the foods allowed. One patient received 18.3 g. of powdered egg white mixed with food so that the biotin was supposedly bound by "avidin." With two exceptions, the daily urinary excretion of biotin exceeded the amount ingested with food, and in all cases the average daily fecal content of biotin was several times that in the diet. As a result, the total biotin excreted each day was approximately three to six times greater than the amount in food. There was no correlation between the biotin content of the feces and the content of the diets used. These data lead to the conclusion that intestinal bacteria synthesize this vitamin and provide a source of the material which is quantitatively of more importance than the dietary source. There was also evidence that the urinary excretion was influenced significantly by the absorption of the synthesized biotin from the intestinal tract. Biotin present in the urine, therefore, comes both from food and from colonic bacteria.

Oppel also observed that the major part of the biotin-like yeast growth promoting substance present in urine behaved abnormally in that it was not bound by egg white or avidin. This was also true for the biotin-like material found in the urine of animals. In contrast, only a small amount of this nonavidin combining growth substance was present in food or feces. No evidence was obtained to indicate that any urinary constituents prevented the avidin-biotin combination. It occurred to the author that the substance might be a product of biotin metabolism, but its excretion was relatively constant even when there were alterations of the diet. Furthermore, the ingestion of biotin concentrates caused an increased excretion of true biotin but did not alter the output of the nonavidin combining growth substance. Pure vitamins and liver or yeast extracts did not influence its elimination. The nature of this "noncombinable biotin," therefore, is not known, but it stimulates the growth of yeast in a manner similar to biotin even in the presence of avidin.

These studies give weight to the accumulating evidence that biotin is synthesized by colonic bacteria and absorbed in quantities sufficient to make man independent of a food source of this vitamin. It is highly improbable that a deficiency of biotin can occur unless the flora of the intestinal tract is drastically altered or unless a highly artificial diet is taken which provides sufficient egg white (or avidin) to bind biotin and prevent its absorption. The investigation also has an academic interest in that it apparently provides the first report of balance studies in man of a vitamin which is provided to a greater degree by the body itself than by food.

DIETARY AND PLASMA PROTEINS

Madden and Whipple (*Physiol. Rev.* **20,** *194 (1940)*) present excellent reasons for abandoning the concept that plasma protein, once formed, is static, barring loss by hemor-

rhage, proteinuria, transudation, or inflammation, and has only physico-chemical and immunologic functions. Protein starved dogs can be maintained indefinitely in a state of nitrogen equilibrium by parenterally administered dog plasma, indicating utilization of such injected protein by the body. Conversely, normal dogs on low protein diets, depleted of their plasma protein by plasmapheresis, draw upon the body's reserve stores for replenishment of the plasma protein. As dietary protein will accelerate the slow regeneration of plasma protein from reserve stores, it would appear that these stores are not in the form of plasma protein nor of material more easily converted into plasma protein than orally ingested protein.

Dogs can be depleted of their stores so that during periods of fasting or protein-free feeding they will produce little plasma protein. Weech (*Bull. Johns Hopkins Hosp.* **70,** *157 (1942)*) produced hypoproteinemia in dogs by three weeks of protein starvation alone. The average rate of decline in the serum albumin during the fourth week on the basal diet was 0.15 g. per 100 ml., in twenty-three experiments. Apparently in protein starvation the body draws upon the reserve stores of protein and upon the plasma proteins for its metabolic needs. Madden and Whipple offer no explanation for the fact that the depleted dog cannot use effectively some of the waste products coming from protein wear and tear or disintegration. "The dog is badly in need of new plasma protein and presumably the stimulus is maximal but the net output of this protein approximates zero when the food protein is zero."

All dietary proteins are not equally effective as precursors of plasma protein. Among the amino acids, Madden and Whipple report that the addition of tryptophane or lysine to the basal diet is ineffective in dogs. Moderate activity in plasma protein formation is exhibited by "histidine + lysine + arginine" and by "cystine + glycine + glutamic acid." Marked activity has been demonstrated for "cystine + glycine + glutamic acid + leucine," with somewhat increased plasma protein formation when "isoleucine + arginine + lysine" or tyrosine was also included. It is likely that other amino acids, present in the basal diet, served to complement those mentioned, in order to produce the observed effects.

Weech determined the relative values of dietary proteins in the regeneration of serum albumin by feeding known amounts to dogs made hypoproteinemic through protein starvation, a technic which is more nearly analogous to conditions of protein starvation in man than the plasmapheresis technic of Madden and associates. However, agreement was obtained in the order of potency values for seven of the eight dietary sources of protein independently tested by both technics. Weech rates lactalbumin much higher than does Madden and Whipple, rating it along with fresh and dried beef serum as a protein of the highest potency. The figures of Madden and Whipple place lactalbumin on a par with beef muscle and liver. The order of potency found for the other seven proteins was, beginning with the highest: beef serum (fresh), beef serum (dried), egg white, beef muscle, liver, casein, and gelatin.

Additional studies by Weech place egg white, whole egg, and cow's colostrum in one group as having similar potencies, second only to beef serum and soluble lactalbumin. Cow's milk, beef chuck, beef liver, and casein are grouped third, although still representing potent precursors of plasma protein. Wheat gluten and gelatin are poor dietary sources of plasma protein and are rated by Weech in the lowest position.

Weech does not report on any other dietary proteins, but Madden and Whipple's report includes estimates on the potency of yeast (fresh, autoclaved), bran flakes, rice polishings, polished rice, powdered Irish potato, salmon, soybean, beef heart (all of which are considered of medium po-

tency, ranking in the order given) and spleen, pork brain, beef stomach, canned salmon, pancreas, and zein (all of low potency).

It would appear from these studies that not all animal proteins are superior to all vegetable proteins in the stimulation of plasma protein formation. (One hundred g. of the protein of either yeast or bran flakes added to the basal diet resulted in the formation of 23 g. of plasma protein, while 100 g. of beef muscle protein resulted in 18 g. of new plasma protein.) It would also appear to be fallacious to consider all "variety meats" as alternates for muscle meats, from the standpoint of the adequacy of their proteins. It is equally apparent that the total amount of protein which must be ingested daily to maintain or replenish the plasma proteins (and reserve stores) will depend upon the potency of the proteins ingested.

The method of estimating the adequacy of dietary proteins by their ability to cause a regeneration of plasma proteins, particularly plasma albumin, has merit and is worthy of more intensive use at this time, when the relative adequacy of both animal and vegetable proteins is a question of national importance.

Madden and Whipple point out that the plasmapheresis technic measures the total capacity to form plasma protein whereas the potency values of Weech measure only relative differences in rate of formation. Nevertheless, the protein starvation method of Weech approximates more closely conditions likely to exist in human malnutrition.

VITAMIN C AND INFECTION

Optimum nutrition probably increases resistance to infectious diseases, as mentioned in a previous discussion of the relation between vitamins and resistance to disease (*Nutrition Reviews* **1,** *66 (1943)*). However, it has been difficult to credit such an effect to any one vitamin or to correlate vitamin levels in an animal with specific immunologic reactions. Perhaps of more practical concern has been the scarcity of critical observations on human beings on a large scale.

Glazebrook and Thomson (*J. Hygiene, Camb.* **42,** *1 (1942)*) reported the results of a study of the effects of ascorbic acid administration on resistance to infection on boys living in a large school in England. The work was done in 1938, at a time when distribution and particularly the serving of food to the 1500 boys (15 to 20 years of age) was poorly managed. Probably as a result of long periods of retention of the meals in hot containers before serving, it was found that the total daily intake of vitamin C for each boy was only 10 to 15 mg. Urinary tests confirmed the serious degree of vitamin C depletion in these subjects, fifteen to twenty-three days being required on a 200 mg. daily intake to reach saturation.

The school population was divided into groups and occupied separate tables in the dining hall. Observations were made on the relation of vitamin C to infection by supplying some of the groups with the crystalline vitamin for several months. Results were treated statistically.

The most frequent infections encountered were the "common cold" and "tonsillitis," the latter being used as an inclusive term for diseases of the nose and throat and included sore throat, otitis media, pharyngitis, and cervical adenitis. There was no difference in the incidence of common colds between the control and vitamin-C treated groups. However, over a six month period, the boys in the control group spent an average of 5.0 days in the infirmary as compared to 2.5 days for the vitamin-treated group. This difference was due to confinement caused by those infections classified as tonsillitis, and

in the incidence of other illnesses. There were 17 cases of pneumonia and 16 cases of acute rheumatism in the 1100 control boys, and not one case of either disease in the ascorbic acid-treated boys, though it should be pointed out that the control group had three times the number of subjects in the group given ascorbic acid.

The results do not justify ascorbic acid therapy in those already receiving "accepted" requirements. In fact, in a later communication, Glazebrook (*Brit. Med. J.* **II,** *617 (1942)*) emphasizes that these results must not be interpreted to mean that vitamin C plays a major role in resistance to disease. He believes that there is a wide variation in the "susceptibility" to hypovitaminosis C among individuals. Thus scurvy may first be indicated by a gingivitis, conditioned by local tissue susceptibility and improper oral hygiene. Likewise anemia may be an early sign of scurvy, but it is conditioned by a simultaneous suboptimal intake of iron. Gingivitis and anemia are said to be often absent in frank scurvy. In his experience, 273 out of 300 individuals with gingivitis and having a low ascorbic acid intake were cured by local treatment only. Twenty-seven required ascorbic acid therapy for complete healing, although the others might have benefited sooner had they also received ascorbic acid. Thus the individual with a latent vitamin C deficiency is placed in a precarious position against the attack of other disease-precipitating factors.

These considerations are undoubtedly important in interpreting the apparent conflict between the observations of Crandon and Lund and the many reports on scurvy from other parts of the world. In the self-imposed scurvy produced by Crandon and Lund, the intake of other dietary factors was adequate and the subject was not exposed to other extraordinary natural conditions. Under these circumstances a severe deficiency of vitamin C was required to produce certain signs of scurvy. Physical signs often associated with naturally occurring scurvy are apparently precipitated by a variety of other conditions not experienced in the more controlled experiment.

Since it cannot be denied that some of our people are subsisting on inadequate diets, including low ascorbic acid intakes, the positive results of Glazebrook and Thomson merit attention by health authorities in their efforts to prevent or lessen the disability caused by infectious diseases.

TISSUE CHANGES IN VITAMIN DEFICIENCIES

When an adequate amount of any of the vitamins fails to reach the body tissues whether because of a defective dietary or poor absorption and utilization, the stores in the tissues gradually decrease, the chemical system in which the particular vitamin is involved becomes defective, function is handicapped, and tissue changes occur. Characteristic tissue change is one of the means by which deficiency diseases are recognized, their response to therapy followed, and the outcome of the deficiency and response to treatment predicted. Therefore, a knowledge of the pathology of the deficiencies is important to an understanding of this type of disease. Wolbach and Bessey (*Physiol. Rev.* **22,** *233 (1942)*) have recently reviewed this subject in an excellent manner. Most of the 345 references cited deal with observations on experimental animals, but because the fundamental pathology is essentially the same in all cases in which the corresponding deficiency occurs in man, these observations are usually applicable to man. In spite of the large amount of work represented by these publications many uncertainties continue to exist in regard to the exact nature

of the tissue changes induced by various deficiencies.

By way of introduction the authors point out that the morphologic changes due to a vitamin deficiency may be peculiar to one type of cell, or, more generally, distributed in several tissues depending upon the particular biochemical process affected and the importance of the process to the cell type. One must endeavor to distinguish between primary effects (the immediate consequences of a deficiency upon tissues in which the vitamin is operative) and the secondary effects (the effects upon the organism as a whole or some other tissue as a consequence of the loss of function of the tissue primarily affected). It is also important not to confuse specific lesions with manifestations of inanition, an almost invariable consequence of any of the deficiencies. Other factors which may affect the morphologic picture are degree and duration of deficiency, and the state of other biologic processes occurring simultaneously, i.e., growth, other deficiencies, and infection.

The effects of vitamin deficiency on the tissue have been divided into four categories. In the first category are grouped the general effects of inanition: retardation of growth of hair, bone, and body as a whole; anemia; and diffuse atrophy of skeletal muscle and various glandular organs. In the second category are placed the effects common to several deficiencies: the peripheral nerve and spinal cord degenerations in thiamine, pyridoxine, riboflavin, and perhaps other deficiencies, and certain nonspecific lesions of the skin. The third category is comprised of more specific degenerative changes: the characteristic skin lesions of nicotinic acid, pyridoxine, pantothenic acid, and riboflavin deficiencies; the various unique degenerations of vitamin E deficiency; and the peripheral nerve lesions of thiamine deficiency. In the final category have been placed the "initial specific effects exhibited by striking changes in structural patterns." The changes in relation to deficiencies of vitamins A, C, and D are cited as belonging to this category. The changes are not primarily degenerative but instead represent either alterations in the cell differentiation (vitamin A deficiency), or changes in developmental sequences (vitamin D deficiency), or a failure in the production of an important structural element (ascorbic acid deficiency).

Of the four categories, the last is perhaps the most interesting and most fundamental. In each deficiency in this category a number of different manifestations have been traced to a unique cytologic aberration. The changes in vitamin A deficiency are of this type. The basic changes in epithelial tissues throughout the body appear to conform to the following rules: (a) only those epitheliums "which have a secretory (chemical) function in addition to the role of a covering layer, and whose functioning cells are without power to divide" are affected; (b) the epithelium atrophies; (c) the basal cells proliferate and produce a stratified keratinizing epithelium which "regardless of the original function . . . is identical in all locations and comparable in all its layers with epidermis;" (d) "following administration of vitamin A, the epithelium of each region returns to its normal type of morphology and function."

In addition to this epithelial change in vitamin A deficiency which is a characteristic lesion in all mammals at all ages, dramatic nerve change may also occur under certain circumstances. If the deficiency is established at a sufficiently early age, skeletal growth becomes retarded in a unique manner before the rate of increase in weight is materially affected. The central nervous system and other soft tissues continue to grow at approximately their normal rate until inanition effects appear. This disproportionate growth of bone and central nervous system leads to overcrowding of the cranial cavity and spinal canal, with subsequent mechanical damage to the brain and spinal nerves.

The morphologic consequences of experimental ascorbic acid deficiency are practically restricted to supporting tissues of mesenchymal origin and may be expressed by the statement that there is failure of formation and maintenance of intercellular materials. This definite and simple characterization explains the pathology of experimental ascorbic acid deficiency in man (scurvy). Hemorrhages, loosening of teeth, failure of wounds to heal and, in infants, extensive subperiosteal hemorrhages and separation of the epiphyses of long bones are manifestations of scurvy and all are explainable on the above simple pathologic principle.

The authors imply that other deficiencies which now present a confused pathologic picture may eventually be explained on the basis of one or at most a few basic tissue changes. On the other hand, vitamins such as riboflavin, nicotinic acid, and thiamine are presumably required in every cell of the body as part of the enzymatic complement. One would, therefore, hardly expect to find the pathologic changes in a deficiency of one of these three vitamins confined to a single tissue or a single type of cell, unless that tissue or cell type had exceptionally high requirements for the vitamin. In general, therefore, in the case of vitamins required by all the tissues of the body, it becomes a competition between the various tissues for the available vitamin, with lesions developing in those tissues that fail to obtain priority of access to the vitamin in keeping with their requirements.

The deficiencies that have been discussed at some length are those of vitamin A, including effects on epithelial tissue, teeth, bone, and nervous system, ascorbic acid, vitamin D, vitamin E, thiamine, riboflavin, niacin, pyridoxine, choline, and pantothenic acid. In addition, the pathologic changes in hypervitaminosis A and D have been described.

The authors conclude with the suggestion that many problems concerning the physiology and pathology of bone, nerve, and skin may be approached through a study of the effects of various deficiencies, since by manipulation of the diet it is possible to produce a variety of specific and reproducible lesions.

DENTAL CARIES IN THE HAMSTER

The albino rat has been the common experimental animal for studies of dental caries. It has been demonstrated repeatedly that caries can be produced in the teeth of these animals by diets containing coarsely ground cereals, but not by diets identical in every respect except that the cereal content is in a finely milled form. The explanations advanced for this phenomenon include abrasion of the enamel by the coarse particles and impaction of food particles on the occlusal surfaces of the molars and between the teeth, leading not only to fractures of the enamel but also to its dissolution by acids formed in the fermentation of the retained carbohydrate. The occlusal surfaces of the molar teeth of the rat are characterized by deep crevices, making them peculiarly susceptible to impaction of food particles.

The production of this disease in the rat is in all probability not strictly analogous to the usual process in man. Arnold (*Pub. Health Reports* **57,** *1599 (1942)*) has succeeded in producing carious lesions in the Syrian hamster, under conditions which make it appear that studies on this animal might provide valuable information concerning the etiology of dental caries in man.

In Arnold's first experiment, 90 animals were divided into three comparable groups at the age of 35 days, and each group was maintained for 90 to 95 days on one of the following diets: (1) corn meal, 66 per cent,

whole milk powder, 30 per cent, alfalfa, 3 per cent, sodium chloride, 1 per cent. The corn meal was coarsely ground, only 3 per cent passing a 60 mesh sieve; (2) the same as (1) except that the corn meal was finely ground, practically all passing through a 60 mesh sieve; and (3) commercially prepared food pellets containing not less than 17.5 per cent protein, 2.5 per cent fat, and 58.0 per cent carbohydrate, and not more than 8.5 per cent fiber. All animals were given carrots or lettuce once a week.

The number of occlusal caries found on the molar teeth and of cervical caries found on the four first molars was reported. Carious lesions occurred in some of the hamsters on all three diets. Sixty-four per cent of the group maintained with "fine corn meal" had occlusal caries and 61 per cent had cervical lesions. Of the animals on "coarse corn meal," 50 per cent had occlusal and 18 per cent cervical caries, while in the third group the respective percentages were 13 and 0. In the animals that had carious teeth the average number of occlusal lesions in the first group was twice as high as in the second, and four times as high as in the third group which received the stock ration.

In a second experiment, Arnold maintained 52 hamsters for 95 to 100 days on a diet in which corn starch was substituted for the corn meal. Fifty-eight per cent of these animals developed occlusal and 46 per cent cervical lesions. This group was composed of 7 litters that demonstrated different degrees of susceptibility. One hundred per cent of the hamsters in one litter developed occlusal caries while only 30 per cent of another litter showed such lesions, suggesting that hereditary, congenital, or early environmental factors are also important in determining the general susceptibility of hamsters to dental caries.

Arnold states that macroscopic examination of the affected teeth revealed indications that the carious process begins in the enamel and proceeds into the dentine (microscopic findings are not reported). Since the susceptible areas on the occlusal surface of the molar teeth are also those favorable to retention of food particles, it is suggested that the lesions result from purely local (oral) causes. Of interest also is the fact that all the experimental and the stock diets contained similar amounts of carbohydrate, yet there was a low incidence of caries in the hamsters on the stock diet. The carbohydrate in the stock diet was provided in pellet form, a form which would provide for a minimum amount of retention of food particles on the teeth.

It may be noted that although the crevices on the occlusal surface of the molar teeth of the hamster are much more shallow and less favorable for impaction of food particles than those of the rat molars, the hamster is more susceptible than the rat to occlusal caries when on diets containing fine cereal particles. Hence, before extending results of animal investigation to human beings it must be appreciated that there are probably great species differences in the susceptibility to caries.

COLOSTRUM AND VITAMIN A

Colostrum milk, that which is excreted immediately following parturition, is particularly rich in various dietary factors. It contains approximately ten times as much vitamin A, three times as much vitamin D and riboflavin, and twice as much ascorbic acid as normal cow's milk. The particular function of colostrum in the nutrition of the newborn, particularly with respect to vitamin A, is beginning to be understood.

Phillips, Lundquist, and Boyer (*J. Dairy Sci.* **24,** *977 (1941)*) have shown that calves at birth are deficient in vitamin A and that this factor is obtained from the colostrum.

The common disease, calf diarrhea, can be controlled often by administration of vitamin A and nicotinic acid. Stewart and McCallum (*J. Dairy Research 13*, **1** (*1942*)) have found that calves born of cows whose colostrum contained less than 250 blue units of vitamin A were more liable to infections such as "white scours" and "navel and joint ills." Their previous studies showed that the vitamin A content of colostrum milks varied greatly and was reasonably independent of the breed of cow, the season of calving, or diet. An attempt was made to increase the vitamin A of the colostrum by feeding 3 pounds of carrots per day to each of 69 cows from the time they were taken off pasture in the autumn until calving time which occurred between November and April. Colostrum samples obtained one hour and twenty-four hours after calving showed no greater vitamin A content than samples collected from 67 control cows receiving no carrots. Another group of cows received 70,000 I.U. of vitamin A per cow per day, again without producing any significant increase of vitamin A in the colostrum milk.

In American farm practice a certain amount of colostrum milk is fed to calves and to fattening pigs, but an estimated 200 million pounds of this valuable milk is thrown away annually. This waste is partially accounted for by the belief that the milk is unfit for human consumption. Colostrum is used by the people of Scotland and Sweden, however, to prepare a pudding which is considered a delicacy. The distinct yellow color and poor consistency of the milk makes it undesirable to mix with commercial milk; hence the practice is prohibited by milk marketing regulations. The problem of utilizing this nutritionally valuable product might well be considered by nutritionists, particularly in these times when every effort should be made to increase the availability of nutritionally superior products.

DIET AND CONGENITAL MALFORMATIONS

Congenital malformations might be caused by faulty nutrition of the fetus, according to occasional assertions in the medical literature. It is easy to understand how faulty implantation of the ovum or abnormalities of the placenta may precipitate these changes, but it has not been entirely established that deficiencies in the maternal diet can also cause fetal malformations. Several studies have claimed that the offspring of animals deficient in vitamin A exhibit certain abnormalities, but these claims have been disputed. Convincing, however, are the reports by Warkany and Nelson (*J. Nutrition* **23**, *321* (*1942*)) that rats bred on a deficient diet frequently give birth to congenitally malformed young.

Thirty-nine per cent of the animals born of mothers fed the deficient ration were abnormal. The diet was composed of yellow corn meal 76 per cent, wheat gluten 20 per cent, chemically pure calcium carbonate 3 per cent, chemically pure sodium chloride 1 per cent. These ingredients were mixed thoroughly and fed *ad libitum* to rats as soon as they had reached the age of 4 weeks. Supplementary vitamin D was administered. The animals grew slowly but frequently attained a weight of 150 g. at 5 to 6 months; regular estrus cycles appeared in the females. The size of the litter born to these animals averaged only 5.2; a greater than normal number of fetuses was resorbed. Malformations were of the following types: shortening of the mandible and protrusion of the tongue, shortening and distortion of the extremities and various degrees of syndactylism of the hands and feet, thinning of the abdominal wall in the ventral line, protrusion of abdominal viscera, reduction in size

of the urogenital papilla, and defective eyes. When animals from the same strain were reared on a stock diet, these abnormalities did not appear and the average size of the litters was 6.82. Addition of 2 per cent dried pig liver to the deficient diet prevented the malformations and increased the number of animals per litter. When the amount of supplementary pig liver was reduced to 0.5 per cent it was no longer entirely protective, but a 95 per cent alcoholic extract of liver was apparently as valuable as whole liver substance. Identical results were obtained with two different strains of rats.

It was observed that the first litter born to a mother on the deficient diet was often normal while subsequent pregnancies yielded a high percentage of deformed fetuses. This suggested that the maternal organism contains enough of the protective factor to see it safely through the first pregnancy, but not enough for subsequent litters unless the diet provides it. Especially interesting is the fact that a female who had been fed the basal ration and who had had abnormal offspring would have a normal litter if she were given the liver supplement prior to her next mating. Conversely, after the normal litter had been delivered, if she were given the deficient diet before being mated again, she would again produce malformed young.

The identity of the alcohol-soluble, protective liver factor is not known. Alfalfa leaf meal does not contain it, at least not in amounts comparable to liver. Because both the mothers and their young reared on the deficient diet showed hyperplasia and hypertrophy of the thyroid, iodine was added as a supplement, but it did not influence the development of fetal deformities. Manganese, deficiencies of which are known to cause abnormalities of embryonic development, was without effect. The authors are now studying the possible protective value of the crystalline factors of the vitamin B-complex, but their data are not complete enough to permit a statement.

One of the most significant contributions of the whole study relates to the time at which the fetal malformations occur during embryonic development and this is presented in a paper by Warkany, Nelson, and Schraffenberger (*Am. J. Dis. Child.* **64,** *860 (1942)*). The gestation period of the rat is twenty-two days and the number of digits becomes recognizable in a fourteen day embryo. If the mothers were fed the deficient diet and given supplements of liver as late as the thirteenth day, the offspring were protected. After the fourteenth day, the addition of liver did not afford complete protection and abnormal fetuses appeared. The nutritional factor in liver, therefore, apparently exerts its effect at about the thirteenth to fourteenth day of the gestation period. The absence or presence of the protective substance at this critical time apparently determines whether or not malformations appear.

These studies by Warkany and associates establish the fact that maternal dietary deficiencies may result in the development of fetal abnormalities. They demonstrate, further, that the protective substance which prevents these changes is contained in liver and in an alcoholic extract of liver. These results cannot be regarded as evidence to show that congenital malformations in man are caused by dietary deficiencies, although they certainly indicate that this possibility should be explored.

THE COLOR OF CANNED PEAS

It is doubtful if any food product is more familiar to the average American than canned peas. Hence it will come as a surprise to many persons to learn that the color of canned peas is not green but olive drab. Canned peas contain no chlorophyll

but a derivative, pheophytin, produced by the loss of magnesium from the chlorophyll molecule. A new method of processing, developed in the laboratories of the American Can Co., conserves the natural pigment and yields a product that is green and tender. A summary of these and many other items of interest is provided in the "Findings of Fact" of the Food and Drug Administration that resulted from hearings held for the purpose of amending the earlier regulations pertaining to the definition and identity of canned peas (*Federal Reg.* **7,** *9918 (1942)*).

When peas are picked from the vine the intensity of their green color begins to decrease, and this diminution in color continues as the peas are shelled and allowed to stand. It is accelerated when they are canned and continues at a lower rate while they are in storage, all because of the gradual conversion of chlorophyll into pheophytin. The change in color can be prevented to a large extent by the new Blair and Ayres process (*Ind. Eng. Chem.* **35,** *85 (1943)*) of canning peas, the development of which led to the request that the Food and Drug Administration revise its definition of identity and list of optional ingredients for canned peas. According to the usual process by which peas are canned, they are shelled, washed, graded, inspected, blanched in hot water, sealed in cans of water containing a little salt and sugar, and finally heated for thirty-five minutes at 240°F. According to the new process the peas are shelled, washed, soaked for about thirty minutes at room temperature in a solution of about 2 per cent sodium carbonate, blanched in a solution of approximately 0.04 per cent calcium hydroxide, washed, and sealed in cans in the usual manner, but in a packing medium which contains about 0.1 per cent magnesium hydroxide. The sealed cans are heated for seven minutes at a temperature of 260°F. when in cans of the size most frequently sold. It is further recommended that the peas canned by this process be stored at a temperature of not over 55°F. Blair processed peas are colloquially known as "greenies" because they have the bright green color of fresh peas, owing to the retention of the natural pigment.

The investigators have not overlooked the nutritional implications of their development. It might be considered that the treatment would result in considerable destruction of thiamine and ascorbic acid, which are readily destroyed in alkaline media. However such is not true when the pH level within the peas is properly maintained in the median pH range (about pH 8) throughout each stage of the canning procedure. It has been found that the range of ascorbic acid content in ordinary canned peas is from 6 to 16 mg. for each 100 g. and, for thiamine, from 0.06 to 0.14 mg. The new product, which at present is more or less of a specialty item, falls within the same range for these two important nutritional components of peas.

Soaking the peas in sodium carbonate solution produces a slight reduction in acidity which is sufficient to retard the change from chlorophyll to pheophytin but it also has a softening effect on the texture of the peas. The addition of traces of calcium hydroxide to the blanch solution retains the alkaline reaction and gives a hardening effect. The magnesium hydroxide which is added in traces to the canning medium provides a reserve of alkali and tends to prevent a change in color during storage. The alkalinity must be kept below pH 8 or undesirable effects are produced. No doubt the change in heat processing to a higher temperature for a shorter period of time helps retain the color and the nutritional quality of the product. The Food and Drug Administration requires that the labeling for this product must bear the statement "Traces of sodium carbonate, calcium hydroxide, and magnesium hydroxide added" or as an

alternative "Traces of alkalies added." Additional analytical work in connection with this investigation is now being prepared for publication.

UNCOMPLICATED THIAMINE DEFICIENCY

One of the objects of animal research in nutrition is to describe the symptomatology and pathology due to the lack of a single vitamin in order to aid in the specific diagnosis of human deficiency diseases. This object has been realized in the case of several of the vitamins due to the great advances in developing purified diets, i.e., diets that are composed of materials whose composition is thoroughly known and that may be supplemented with pure vitamins. In the case of thiamine, however, many diets used to produce a deficiency of this factor have contained autoclaved yeast to supply other vitamin B-complex factors. Recent work casts suspicion on the assumption that such autoclaved yeast supplies enough of all the necessary B-complex factors to protect the animal against deficiencies other than thiamine deficiency.

The question in point is whether thiamine deprivation is responsible for nerve degeneration. There is no question that acute thiamine deficiency produces neuropathologic disturbances and that crystalline thiamine administration alleviates these symptoms in a short time. This has often been termed a "functional" pathology since a morphologic change is by no means a constant finding in this condition. It is common to associate chronic thiamine deficiency and nerve degeneration, based upon observations in human beriberi. It is of course unwarranted to believe that an individual showing beriberi has selected a diet deficient only in thiamine. The specificity of the relation of chronic thiamine deficiency to nerve degeneration is questioned by the findings that lack of riboflavin produces degeneration of the myelin of the main peripheral nerve trunks, and that pantothenic acid and pyridoxine are also involved in the prevention of sensory neurone degeneration in the growing pig.

The earlier work of Van Etten, Ellis, and Madsen (*J. Nutrition* **20,** *607 (1940)*) suggests that substances other than thiamine might be destroyed in the autoclaving of yeast. Swine were fed a diet that contained liver and whey as chief sources of the B-complex. When the diet was treated with sodium sulfite and sulfur dioxide, a procedure which, as far as we know, should destroy only the thiamine, a deficiency syndrome resulted that was remarkable in that neuropathologic signs were absent. If, however, the same diet was autoclaved, the animals developed convulsions and nerve degenerations that were not unlike those ascribed to pyridoxine and pantothenic acid deficiency.

More recently Wintrobe and co-workers (*Bull. Johns Hopkins Hosp.* **71,** *141 (1942)*) found that young pigs could be raised satisfactorily on a simple diet, somewhat deficient in the B-vitamins, but which was supplemented by pure thiamine, riboflavin, nicotinic acid, pyridoxine, choline, and pantothenic acid. Although their growth data indicate that the diet may not be entirely complete, it is a good approach to a synthetic thiamine-deficient diet.

All the experimental and control animals received B-vitamins in the form of brewers' yeast from about 3 weeks of age until they were selected for study, at ages ranging from 69 to 90 days. Then the brewers' yeast was withdrawn and the crystalline vitamins were substituted. Upon removal of thiamine, the chief symptoms developed were anorexia, vomiting, dyspnea, cyanosis, and great weakness. Symptoms of cardiac failure appeared suddenly and, unless thiamine was given, resulted promptly in death.

Unexpected death occurred in some instances without dyspnea and cyanosis having been observed. Focal necroses of the myocardium were observed in all animals dying of thiamine deficiency. No neurologic symptoms were observed, nor were any degenerative changes in the nervous system found, even in those animals with thiamine deficiency of as long a duration as 320 days.

There is little doubt that a thiamine deficiency was actually produced. There occurred a decreased thiamine excretion in the urine, with or without a test dose, an elevated blood pyruvate and a prolonged elevation of pyruvate after feeding glucose. The measure of blood pyruvate after glucose ingestion was considered better than a single basal determination; both were correlated with the onset and severity of the symptoms of thiamine deficiency.

These studies indicate strongly that, if there are no great species differences, the neuropathologic syndrome previously attributed to chronic thiamine deficiency may actually be due in large part to a lack of other dietary essentials.

SPECIAL DIETARY FOODS AND THE LAW

A new trend in offering food to the public was recognized by Congress in 1934 when, at the suggestion of the Food and Drug Administration, a bill was introduced in the Senate which provided, among other things, the regulation of foods represented for special dietary uses. Under this bill, special emphasis was given to the nutritive quality of food.

In recommending approval of an amended version of this bill, the Committee said: "Paragraph g deals with articles offered for special dietary uses, such as infant foods, invalid foods, slenderizing foods and other dietary products intended for special nutritional requirements. It authorizes the establishment of regulations, subject to the approval of Committee on Public Health provided by Section 22, requiring statements concerning vitamin, mineral or other properties needed for the intelligent use of the food by the consumer. The science of nutrition is rapidly extending the field of its usefulness. In order to keep abreast of these developments, it is necessary that regulation-making power be given here. It is particularly essential in the field of baby foods."

It was not until June, 1938, that this bill (there were 27 different versions before it was enacted into a law) was approved by Congress. This law is known as the Federal Food, Drug and Cosmetic Act. Section 403 J provides: "A food shall be deemed to be misbranded if it purports to be or is represented for special dietary uses, unless its label bears such information concerning its vitamin, mineral and other dietary properties, as the Federal Security Administrator determines to be, and by regulations prescribes as, necessary in order to fully inform purchasers as to its value for such uses." Under the authority of this section, the Federal Security Administrator, Paul V. McNutt, promulgated regulations for food represented for special dietary uses, which became effective May 17, 1942. In a statement accompanying the announcement of these regulations, Mr. McNutt said: "The requirement of the Act that fully informative labeling, as specified in regulations, appear on foods for which special dietary claims are made is squarely in line with the national program for better nutrition. The appearance of such labeling on foods having unusual dietary values, will, I believe, stimulate the interest of consumers in their dietary needs and lead to a more intelligent planning of their daily meals. The educational program for better nutrition now being conducted as an essential to National Defense will be furthered by label state-

ments that are factual and informative, but not exaggerated. The labeling requirements prescribed by the regulations are not burdensome and will not handicap the marketing of foods which have merit for special dietary uses."

At the same time, the Federal Security Administrator promulgated regulations which defined the term "special dietary use" to mean particular (as distinguished from general) uses of foods, such as (1) uses for supplying particular dietary needs which exist by reason of physical, physiologic, pathologic, or other conditions, including but not limited to the conditions of disease, convalescence, pregnancy, lactation, allergic hypersensitivity to food, underweight, and overweight; (2) uses for supplying particular dietary needs which exist by reason of age, including but not limited to the ages of infancy and childhood; (3) uses for supplementing or fortifying the ordinary or usual diet with any vitamin, mineral, or other dietary property. Any such particular use of a food is a special dietary use, regardless of whether such food also purports to be or is represented for general use.

The regulations provide that the label of a food represented for a special dietary use shall bear a statement of the dietary properties upon which such use is based, and that such statement shall show the presence or absence of any substance, any alteration of the quantity or character of any constituent, or any other dietary property upon which such use is based. If the special dietary use is by reason of its vitamin property in respect to vitamin A or its precursors, vitamin B_1, vitamin C, vitamin D, or riboflavin, or by reason of its mineral property in respect to calcium, phosphorus, iron, or iodine, the label shall bear a statement of the proportion of the minimum daily requirements for such vitamin or mineral property supplied by such food when consumed in a specified quantity during a period of one day. Exceptions are made in the case of cow's milk and evaporated milk with respect to the declaration of vitamins and in the case of iodized salt with respect to the declaration of iodine. The specified quantity shall be the quantity customarily or usually consumed during the period of one day or a quantity reasonably suitable for consumption within such period.

Where the purported special dietary use is in respect to any vitamin or mineral property not listed in the regulations, the label must bear a statement of the quantity of such vitamin or mineral element in a specified quantity of such food. Where the need in human nutrition for such vitamin or mineral element has not been established, the label must also bear the statement: "The need for ———— in human nutrition has not been established."

Where a food is represented for treating any disease resulting from a dietary deficiency of any vitamin or mineral element the label must bear a statement of the quantity of such vitamin or mineral element in a specified quantity of food, and must also bear adequate directions for use.

For the purposes of the regulations, the minimum daily vitamin and mineral requirements are:

Vitamin A —1500 U.S.P. units for an infant
3000 U.S.P. units for a child
4000 U.S.P. units for an adult

Vitamin B_1 —0.25 mg. for an infant
0.5 mg. for a child under 6 years of age
0.75 mg. for a child 6 or more years old
1.0 mg. for an adult

Vitamin C —10 mg. for an infant
20 mg. for a child
30 mg. for an adult

Vitamin D —400 U.S.P. units for an infant, child, or adult

Riboflavin —0.5 mg. for an infant
2.0 mg. for an adult

Calcium —750 mg. for a child or an adult, except a pregnant or lactating woman, in which case the requirement is 1.5 g.

Phosphorus—750 mg. for a child or an adult, except a pregnant or lactating woman, in which case the requirement is 1.5 g.

Iron —7.5 mg. for a child less than 6 years old, 10 mg. for a child 6 or more years old or for an adult, except a pregnant or lactating woman, in which case the requirement is 15 mg.

Iodine —0.1 mg. for a child or an adult

The above quantities were based upon estimated intakes that would protect individuals from clinical symptoms of the respective deficiency diseases; hence they differ from the National Research Council's "Recommended Daily Allowances."

There are special label requirements for food represented for special dietary use for infants. Such label must bear the common or usual name of each ingredient, including spice, flavoring, and coloring. If such a food consists in whole or in part of plant or animal matter, the label must reveal the specific plant or animal which is its source. If the food simulates human milk or purports to be a complete or partial substitute for human milk, the label must also bear (a) a statement of the per cent by weight of moisture, protein, fat, available carbohydrate, crude fiber, calcium, phosphorus, and iron contained in such food; (b) a statement of the number of available calories and U.S.P. units of vitamin A, vitamin B_1, vitamin C, and vitamin D supplied by a specified quantity of such food; and (c) a statement that an additional quantity of vitamin or iron, as the case may be, should be supplied from other sources, if less than 30 U.S.P. units of vitamin C, or less than 50 U.S.P. units of vitamin D, or less than 0.75 mg. of iron, is supplied by the quantity of such food which, as customarily or usually prepared for consumption, supplies 100 available calories. The provisions of (a) and (b) do not apply to cow's milk and evaporated milk. Provision (c), with respect to vitamin D, does not apply to cow's milk or evaporated milk in which the vitamin D is increased by any means to not less than 135 U.S.P. units to each quart in the case of cow's milk and not less than 7.5 U.S.P. units to each avoirdupois ounce in the case of evaporated milk.

Special labeling requirements are made for food represented for use in control of body weight or in dietary management with respect to disease. Such a food must bear a statement of the per cent by weight of protein, fat, and available carbohydrates in such food and the number of available calories supplied by a specified quantity of such food.

There are special labeling requirements for a food purported to be for special dietary use by reason of the presence of any constituent which is not utilized in normal metabolism. In such cases the label must bear a statement of the per cent by weight of such constituent and the word "non-nutritive." Where the constituent is fibrous plant matter, it shall be considered to be crude fiber, and its per cent expressed as such. But if such constituent is saccharin or a saccharin salt, the label must bear the statement "Contains ———— saccharin [or saccharin salt, as the case may be] a non-nutritive artificial sweetener which should be used only by persons who must restrict their intake of ordinary sweets," the blank to be filled in with the per cent by weight of saccharin or saccharin salt in such food. This provision authorizes the use of saccharin or saccharin salt only in food intended for persons who must restrict their intake of carbohydrates.

Special labeling requirements are also made for food represented for special dietary use by reason of the decrease or absence or any allergenic property. Such foods must bear (a) the common or usual name and the quantity or proportion of each ingredient, including spices, flavoring, and coloring, and (b) a qualification of the name of the food or of the name of each ingre-

dient, to reveal clearly the specific plant or animal which is the source of such food or such ingredient, if such food or ingredient consists in whole or in part of the plant or animal matter, and the name does not clearly reveal the specific plant or animal which is such source, and (c) a statement indicating the nature and effect of any treatment or processing of the food or any ingredient thereof, if the changed allergenic property results from such treatment or processing. The Journal of the American Medical Association, in an editorial on these regulations, (*J. Am. Med. Assn.* **117,** *2170 (1941)*) said in part: "The public should know the nutritional properties of the food it purchases. When these regulations become effective, this information will be provided for the first time in terms that can be understood. The statement on a label that an article contains 1 mg. of riboflavin means little to the average purchaser; but when the label states, as it will under the new regulations, that a specified quantity of cereal X contains one-half the minimum adult daily requirement of riboflavin the purchaser knows at least that additional riboflavin is needed. The Food and Drug Administration is to be commended for a job well done."

CITRIN AND RELATED PRODUCTS

In 1936 Szent-Györgyi reported a cure of a case of hemorrhagic diathesis by an extract prepared from paprika. Although this preparation apparently contained ascorbic acid, the curative effect was not attributed to this vitamin since another similar patient treated with pure ascorbic acid showed no clinical improvement. Assuming an identity of the curative factor with substances which he had found active in a plant oxidation system, Szent-Györgyi prepared a mixture of substances from lemons which he called "citrin." This mixture appeared to consist essentially of two compounds, hesperidin and its derivative eriodictin, which are flavones by chemical classification.

From their studies of citrin, the Hungarian workers reported that much of the hemorrhagic manifestation of scurvy in guinea pigs was actually due to lack of citrin in the experimental diet. Several reports decisively discredited the citrin effect in experimental scurvy, and Szent-Györgyi himself was unable to justify his earlier reports. Meanwhile the name vitamin P, to connote its supposed necessity in the diet and its effect on capillary permeability, had been applied to citrin. Neither of the two postulates from which the name was derived was adequately supported.

Because of the great clinical interest in hemorrhagic conditions, several clinic groups were stimulated to test the effect of citrin preparations in a variety of disease conditions. Several of these reports have dealt only with clinical improvement, but others have attempted to correlate citrin or hesperidin effects with specific hemorrhagic conditions and capillary resistance changes. Beneficial effects have been reported in purpuras of various etiologies, vascular, infectious, and allergic; in psoriasis; and in renal, intestinal, and gum bleeding. Most of these reports include reference to earlier unsuccessful use of ascorbic acid in the same patients or in a separate group, but do not offer convincing evidence that the disease conditions treated were due to a dietary lack or that citrin belonged in the classification of a vitamin. Furthermore, question has been raised as to the adequacy of capillary fragility tests and to the types of preparations used by the different investigators. In the absence of an experimental test for production of the supposed deficiency, and lacking even an acceptable criterion for its

detection, it was impossible to evaluate or compare the results in a satisfactory manner.

More recent reports fall into the category of those which must be seriously considered as evidence for the existence of a new vitamin. Scarborough and co-workers (*Biochem. J.* **33,** *1400 (1939)); Lancet* **II,** *610, (1938)); and Ibid.* **II,** *644, (1940))* found that in 7 patients with generalized vitamin deficiency, the condition of decreased capillary resistance was not altered by various vitamins, including ascorbic acid, until hesperidin or citrin was administered. In one study, 5 patients who had been diagnosed as having scurvy were kept on a vitamin-deficient diet and fed various vitamin supplements. The administration of ascorbic acid cured the gingival bleeding as well as the subcutaneous and muscular hemorrhage, an effect not accomplished by "vitamin P" preparations. In spite of this effect of ascorbic acid, the capillary resistance of the subjects remained low and only after the administration of citrin was there a prompt increase in resistance. In 2 of the patients maintained on the basic diet, and in spite of continuous ascorbic acid treatment, there was a recurrence of low capillary resistance. Scarborough did not however, report the parallel or comparable change in the capillary resistance of a control group on the same diet. He concluded that there is a differentiation between the spontaneous hemorrhage of scurvy and decreased capillary resistance, and that lack of consideration of this fact had led to the conflicting reports on the action, and indeed the existence, of "vitamin P."

From the standpoint of experimental production of the deficiency, the Hungarian workers St. Rusznyák and Benkó (*Science* **94,** *25 (1941))* confirm Zacho's earlier report (*Acta path. et. microbiol. Scandinav.* **16,** *144 (1939))* that the "specific" symptom of capillary fragility is the best index of the "vitamin P" effect. They reported also that rats, which do not develop scurvy, nevertheless developed an increased capillary fragility when fed a typical scorbutogenic diet. This is an important finding in the differentiation of the known capillary changes during scurvy from those of citrin deficiency, but many other laboratories working with purified diets in rat feeding experiments have not reported any capillary changes.

Bacharach, Coates, and Middleton (*Biochem. J.* **36,** *407 (1942))* have devised a biologic test for the purpose of measuring "vitamin P" activity. Young guinea pigs of a limited weight range were placed on a scorbutogenic diet consisting of bran, crushed oats, and separated milk powder, supplemented with cod liver oil and 5 mg. of pure ascorbic acid daily. The test preparations were also given by mouth. Recrystallized hesperidin (difficultly soluble) was given in aqueous suspension, and a second preparation containing a mixture of glycosides from "citrin," together with calcium salts, was administered in aqueous solution. The latter was prepared from orange peel and pulp.

The animals were prepared for testing by shaving the skin over a 2 cm. wide area on either side of the lumbar vertebrae. The skin was treated with vaseline and the animals were placed in a warm room to insure a good blood supply to the region and to facilitate observation of petechial hemorrhage. A suction cup with a glass window was applied to the area and the pressure gradually lowered. The negative pressure, recorded by a connecting manometer, was noted when petechial hemorrhages were first seen. This value is called the "critical petechial pressure." The pressure was reduced by 5 mm. intervals and a 3 to 5 second interval was allowed for observation at each pressure. The variation of these values with such factors as exact position of the suction cup and differences between observers is claimed to be unusually small. The average critical petechial pressure at the time of beginning the test was 95 mm. of mercury. On the test diet the critical

petechial pressure decreased some 35 to 40 mm. of mercury in two to three weeks. A group of animals receiving 4 mg. per day of the water soluble mixture with calcium, maintained an approximately steady critical petechial pressure. Those receiving 5 to 20 mg. of hesperidin per day showed some protection with increasing dosage, but none maintained their original values as a result of this supplement.

Certain difficulties were pointed out in the use of the method. It is suggested that changes in the environmental temperature or the presence of extravascular blood in any location of the body may lead to increased capillary resistance. Animals that were cut during shaving maintained an abnormally high capillary resistance, and it is probable that the hemorrhage coincident with scurvy would itself protect against a lowering of capillary resistance. Whether or not this becomes a useful assay method, the results at least provoke thought as to the existence of a factor in the glycoside preparation which is active on capillary resistance, possibly of a vitamin nature.

From the chemical standpoint, Wawra and Webb (*Science* **96,** *302 (1942)*) have recently isolated a crystalline derivative of hesperidin from lemon peel. This substance, yellow in color, and classified as hesperidin chalcone, is derived from hesperidin by opening of one of the rings in its molecular structure. The chalcone can be readily reduced to hesperidin and the latter reoxidized by air. It was mentioned that preliminary experiments showed that "the chalcone exerts a beneficial effect upon the state of the capillaries, decreasing the fragility and preventing localized hemorrhages." Hesperidin chalcone, by virtue of its reversible oxidation and reduction, was found to be active catalytically in the isolated glutamic acid oxidation system of liver. The authors therefore suggest that the compound may play an active part in tissue respiration of both plants and animals. However, other reversibly oxidizable dyes can also play a catalytic role in the enzyme system tested, so that the natural functioning of the compound is only indicated as a possibility.

DIETARY FACTORS IN CANCER TISSUES

Experimental epidermal carcinomas are easily induced in mice by means of carcinogenic hydrocarbons such as methylcholanthrene. Topical applications of this compound cause early changes in epidermis and upon repetition of the treatment well defined carcinomas develop. Carruthers and Suntzeff (*J. Nat. Cancer Res.* **3,** *217 (1942)*) and Wicks and Suntzeff (*J. Nat. Cancer Res.* **3,** *221 (1942)*) used methylcholanthrene to study the chemical, histologic, and physical changes in skin as the carcinogenic process advanced.

The mice were prepared for experimentation by closely shaving the skin of the back and then painting the area with a single stroke of a brush. The controls were painted with reagent-grade benzene and the experimental animals were painted with 0.6 per cent benzene solution of methylcholanthrene. Determinations were made at appropriate intervals for total iron, ascorbic acid, and total lipids.

Studies on cancer tissues, whether concerned with composition or with enzyme activity, have long been complicated by lack of a suitable "normal" tissue with which to compare the results obtained. In the case of skin, for instance, the tumor arises from specific cells in the basal layer, and there is no assurance that these cells, even in the normal state, do not differ considerably from the average composition of the entire skin. The skin of the normal mouse consists of two undifferentiated layers. When a single application of the

hydrocarbon is applied, the skin differentiates into at least four layers. Hence the St. Louis investigators used nucleoprotein phosphorus as the basis for comparing their results on the determinations of iron and ascorbic acid. This was interpreted to represent the number of viable cells more truly than such measurements as the percentage of dry weight.

A single application of methylcholanthrene reduced the total iron content of the epidermis of the mice within a few days to approximately 50 per cent of its normal content. Multiple applications of the carcinogen applied on alternate days caused a further lowering. Ascorbic acid was not significantly altered during the course of epidermal carcinogenesis. The ascorbic acid content if calculated on a weight basis was significantly reduced, but when the ratio of ascorbic acid to nucleoprotein phosphorus was used as the base line instead of tissue weight the ascorbic acid content was the same in all groups. The total lipid material extractable from the epidermis of mice was found to be much lower a few days after even a single topical application of methylcholanthrene. Mice treated with benzene showed no such reduction. As a standard of reference for the determination of the total lipids of skin, protein nitrogen values were used and interpreted to represent the total protoplasm present.

The results suggested that both iron and lipid metabolism might be involved in carcinogenesis. Since the carcinogens are soluble in fat and insoluble in water an interference with lipid metabolism would readily be possible. The connection with iron is less obvious, although cytochrome, an iron-containing respiratory enzyme, is known to be low in tumor tissue. Further studies are needed to determine the effect of the ingestion of iron and lipids upon the progress and growth of epidermal cancers, and to correlate the development of carcinomas with other chemical constituents of the tissue.

IRON AND THE VITAMIN B-COMPLEX IN MAN

The impression has grown that an intimate relationship exists between the B-vitamins and iron absorption or utilization. It has been pointed out that some patients with pellagra develop an iron deficiency or hypochromic anemia. György, Robscheit-Robbins, and Whipple (*Am. J. Physiol.* **122,** *154* (*1938*)) have shown that riboflavin increases the rate of hemoglobin production by the anemic dog. Gastrointestinal abnormalities incident to avitaminosis may possibly lead to defective absorption of iron. Pyridoxine deficiency in experimental animals has been shown by Fouts, Helmer, Lepkovsky (*Am. J. Med. Sci.* **199,** *163* (*1940*)); Wintrobe and associates (*Bull. Johns Hopkins Hosp.* **72,** *1* (*1943*)); and others to cause hypochromic anemia because it interferes with iron utilization. This last demonstration, however, is the only clearcut evidence that an interrelationship does exist between iron and any component of the B-complex. It obviously does not imply that the B-vitamins have any therapeutic value in the treatment of hypochromic anemia in man. Yet, because of these data, and because of the belief that subcritical deficiencies of the vitamin B-complex are relatively common in the United States, many physicians have come to treat their patients with hypochromic anemia by giving them the relatively expensive combinations of iron and the B-vitamins.

In order to determine the validity of these impressions and therapeutic practices, Moore, Minnich, Vilter, and Spies (*J. Am. Med. Assn.* **121,** *245* (*1943*)) studied 50 adult patients with hypochromic anemia who were observed in the Nutrition Clinic

of the Hillman Hospital in Birmingham. With but few exceptions, all these individuals had had recurring deficiency disease for years. At the time of the study, 23 (46 per cent) showed objective clinical evidence of niacin deficiency, 20 (40 per cent) had ariboflavinosis, 7 (14 per cent) had peripheral neuritis, and 18 (36 per cent) showed no recognizable evidence of deficiency. These figures total more than 100 per cent because two or all three types of deficiency were present in 15 of the patients. It was noted, however, that the incidence of each type of deficiency disease among these subjects was roughly the same as among all the patients visiting the clinic. Furthermore, instances of macrocytic and normocytic anemia were likewise found in patients with each type of deficiency. There was, therefore, no tendency for hypochromic anemia to occur specifically with niacin, riboflavin, or thiamine deficiency.

The response to therapy was likewise studied. Ten patients with hypochromic anemia and deficiency disease were given iron orally. No other medication was permitted, vitamins of the B-complex were rigidly avoided, and the subjects were instructed to make no changes in their diets. In each instance when iron was given, a reticulocyte response occurred. The average rate of hemoglobin regeneration was as rapid as is usual when specific therapy is given to individuals with iron deficiency anemia. The lesions of the deficiency disease usually remained relatively stationary during the period of iron therapy and responded in characteristic manner when niacin, riboflavin, and thiamine were subsequently administered. To 4 additional subjects, 75 g. of brewers' yeast were given together with iron after a preliminary two week period during which iron alone had been allowed. None of these patients developed a secondary reticulocyte response, and the rate of hemoglobin regeneration was no more rapid than it had been in the patients treated with iron only. There was, therefore, no evidence that the absorption or utilization of iron was impaired in any of the subjects with deficiency disease.

Particular attention was given to the possibility that some of the patients might have had a pyridoxine deficiency. However, none of the subjects failed to respond to the therapeutic administration of iron, and pyridoxine administration alone did not stimulate hemoglobin regeneration. Furthermore, experimental animals with pyridoxine deficiency have a high level of serum iron rather than the low level found in states of iron depletion. All the individuals studied in this investigation had low serum iron values. If pyridoxine deficiency occurred among these patients, therefore, it was not characterized by hypochromic anemia and high serum iron values.

Since evidences of chronic hemorrhage were detected in nearly all the subjects with hypochromic anemia, the authors conclude that blood loss, rather than the avitaminosis, was the etiologic factor responsible for the development of the iron deficiency.

Vitamins in general, and the B-vitamins in particular, have been related to many of the functions of living tissue. It is not surprising, therefore, that enthusiasts may on occasion assign to these substances functions which they do not have. This seems to be the case for the B-vitamins as they affect iron metabolism in man. For until pyridoxine deficiency is demonstrated in human subjects, or until new interrelationships are discovered, it must be concluded that there are no known, direct influences of the B-complex on iron metabolism in man. Moore and associates have certainly shown this to be true for niacin, riboflavin, and thiamine. The current practice of treating hypochromic anemia with both iron and the B-vitamins would seem to be an unnecessary form of dual therapy.

FURTHER EXPERIMENTS ON THIAMINE DEFICIENCY IN MAN

The signs and symptoms of mild thiamine deficiency in man are vague, in that the abnormalities reported and observed cannot be considered as specific indications of that deficiency disease; they occur in disease conditions of different etiology. They are the little signs and symptoms of malnutrition that might be experienced by presumably healthy persons who are able to perform their usual daily routine. Certainly even the best nourished members of the human race do not go through life with an ever-present feeling of buoyant health, nor is it likely that the most enthusiastic investigator in nutrition would claim that such a Utopian condition is possible. On the other hand there is no doubt that the symptoms of malaise are real and important to the person who is experiencing these phenomena.

It is significant that further research corroborates in the main the principal findings of earlier investigators of induced thiamine deficiency in man. Confirming in general the observations briefly reported in this journal (*Nutrition Reviews* **1,** *46* (*1942*)), Egaña and collaborators at Harvard University (*Am. J. Physiol.* **137,** *731* (*1942*)) report the results of a study made of 7 men between the ages of 27 and 42 years who were maintained on a thiamine deficient diet. The subjects of the experiments were physicians engaged in sedentary work, and the ability of the men to undergo physical exercise was carefully measured. The results showed definitely the importance of an adequate thiamine intake for persons doing manual work, even of a moderate nature.

Each subject was allowed unlimited amounts of foods that are low in the vitamin B-complex. These consisted of ordinary white bread, soda crackers, butter, cheddar cheese, macaroni, spaghetti, polished rice, farina, heavy cream, sugar, honey, molasses, tapioca, salad oil, egg white, ice cream, puffed rice, coffee, tea, salt, pepper, vinegar, hard candies, and gelatin. Small amounts of orange juice, grapefruit juice, onions, and lettuce were given each day and twice a week very small portions of meat, fish, or poultry were permitted. Supplements of halibut liver oil supplying 8500 I.U. of vitamin A and 1700 I.U. of vitamin D, as well as 50 mg. of ascorbic acid were given. The diet was considered adequate in riboflavin but deficient in thiamine and it probably was somewhat deficient in nicotinic acid, pantothenic acid, and pyridoxine. At the end of the experimental period each subject was given 36 g. daily of a brand of dried brewers' yeast which supplied from 7.2 to 10.8 mg. of thiamine, about 16 g. of protein, and significant amounts of riboflavin, pantothenic acid, and pyridoxine.

The suitability of the diet was shown by analyses of the urine for thiamine and also by the performance of thiamine saturation tests at the beginning and end of the deficiency period. The protein intake was estimated by determination of the urinary nitrogen excretion and it was considered nutritionally satisfactory. The caloric intake was considered to be adequate in 3 of the subjects and somewhat low in the others, as shown by a slight loss of weight.

The deficient diet was continued for periods up to four weeks. In this time no striking symptoms were reported but 5 of the subjects noted that they were not feeling well and complained of easy fatigue and loss of efficiency in daily work; 3 complained of forgetfulness; constipation was noted by one, irritability by 2, paresthesia by 2, slight nausea or diarrhea by 2, and muscle and joint pains during motion by one. Although none of the subjects felt completely well, none was ill or debilitated. There was some loss of ability for performing exhausting exercise and in particular there was poor recuperation between periods of such exer-

cise. All these changes were reversed by adding brewers' yeast to the diet.

Little or no change was noted in the metabolic measurements that were made. The measurements consisted of determinations of oxygen consumption, carbon dioxide loss, concentration of pyruvic acid and sugar in the blood, and the amount of lactate and pyruvate excreted in the urine. Of interest were the observations on the cardiovascular system. No tachycardia on exertion was noted; instead the heart rates tended to be abnormally low both in normal exercise and in exhausting exercise. There were no unusual changes in blood pressure and in only one subject did the electrocardiograph show any significant change. The authors have pointed out that the only reliable signs of deficiencies under conditions such as those which prevailed in their experiments are the determination of the amounts of vitamins found in the urine and the little symptoms and signs suggesting deterioration of the whole organism.

Although the authors believed that the diet contained sufficient riboflavin they also mentioned that 2 subjects developed noticeable cheilosis and scaling at the angles of the nose, conditions which cleared up after taking yeast. These signs are usually observed in riboflavin deficiency. It is also possible that the diet was deficient in calcium and phosphorus. The administration of yeast would relieve deficiency of phosphorus and of the factors of the B-complex other than thiamine, but it would not relieve a deficiency of calcium.

The striking feature of the experiments of the Harvard investigators is the rapidity with which the signs of deficiency were developed. Their observations afford additional support to the new concept of thiamine deficiency that differs considerably from the older idea of beriberi as the condition which results from lack of vitamin B_1. The effect of thiamine deficiency on the ability to perform work is of immediate interest in the war effort. Psychiatrists should not overlook the interesting personality changes that repeated studies have shown to result from moderate deficiency of thiamine in the diet. Would further investigation show that some—certainly not all—of the personality problems of human beings have a basis in unfortunate food habits that prevent the securing of an adequate diet?

SYNTHESIS OF VITAMINS IN THE DIGESTIVE TRACT

Twenty years ago Steenbock, Sell, and Nelson (*J. Biol. Chem.* **55,** *399 (1923)*) and Dutcher and Francis (*Proc. Soc. Exp. Biol. Med.* **21,** *189 (1923–24)*) called attention to the fact that the ingestion of excreta by rats, subsisting on a so-called vitamin B-free diet, led to cessation of vitamin B deficiency symptoms, accompanied by increased growth. The prevention or diminution of coprophagy (feces eating), by the use of screen bottomed cages, led to the usual evidence for vitamin B deficiency. Deficiency symptoms disappeared and growth was resumed by the introduction of feces or by feeding vitamin B-containing foods. Heller, McElroy, and Garlock (*J. Biol. Chem.* **65,** *255 (1925)*) confirmed these findings and presented evidence to show that the vitamin B content of feces has its origin in spore bearing organisms which flourished in the intestinal tract.

In the years that followed, laboratories here and abroad added additional evidence to show that coprophagy tends to vitiate the accuracy of vitamin B assays and that bacterial synthesis in the digestive tract is partly responsible for varied results obtained. In searching for a solution to the problem, the Pennsylvania workers studied different types of carbohydrates, with the thought that these might affect the intestinal flora to the point where vitamin syn-

thesis by intestinal organisms might be affected. Coprophagy appeared to have little effect when sucrose was used as the sole source of carbohydrate, while serious errors were often observed when dextrinized corn starch was used.

Since the type of carbohydrate appeared to cause such pronounced differences in growth studies with coprophagous rats, additional types of carbohydrates were studied by Guerrant, Dutcher, and Tomey (*J. Biol. Chem.* **110,** *233* (*1935*)). These studies included testing sucrose, glucose, lactose, raw corn starch, and dextrinized corn starch as the sole source of carbohydrate in the various experimental groups. Excreta of various groups were interchanged so that each group received excreta from all other groups, in addition to that produced by the group itself. Feces of rats receiving dextrinized corn starch were most potent in the vitamin B-complex, while little or no evidence was found to show that glucose or sucrose supported vitamin synthesis. Fecal pellets from rats fed lactose or raw starch showed some evidence of vitamin synthesis.

When ceca were removed, rats would not grow, even when dextrinized corn starch was fed. Good growth was obtained when vitamin B-complex was added to the diet of cecectomized animals or when these animals received feces which had been voided by rats which had not been operated upon and which had received the vitamin B-free diet containing dextrinized starch.

The cecum, it was concluded, is the principal site of vitamin synthesis, and microorganisms, especially wild yeasts, multiply in the cecum, using available carbohydrates as sources of energy. Glucose and sucrose appeared to be absorbed so rapidly in the upper intestine that little or no carbohydrate was available for the multiplication of cecal microorganisms. However, considerable dextrinized corn starch was always present in the distended ceca, accompanied by high wild yeast counts. It should be pointed out that vitamins thus synthesized are not available to the rat in appreciable amounts except through coprophagy. Griffith (*J. Nutrition* **10,** *667* (*1935*)) conducted similar experiments and concluded that bacterial synthesis of vitamin G (B_2) takes place in the digestive tract of cecectomized animals and that the cecum is not the principal source of bacterial synthesis. He did observe that cecectomized animals grew better when they were permitted to resort to coprophagy. However, it should be borne in mind that his rats subsisted on a ration which contained 75 per cent of sucrose, which does not favor optimal development of microorganisms in the cecum.

Light, Cracas, Olcott, and Frey (*J. Nutrition* **24,** *427* (*1942*)) have endeavored to determine whether the depressed growth observed in rats when sulfaguanidine is fed, is due to the effect of the drug on the metabolism of the rat or to the prevention of "a symbiotic synthesis of unidentified factors by the intestinal flora." The inclusion of 0.5 per cent sulfaguanidine in a diet which contained all the available crystalline factors (thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, choline, and biotin) resulted in depressed growth. Additions of yeast or yeast extracts counteracted this growth-depressing effect. Growth loss was also prevented if sulfaguanidine fed rats were given feces from rats which received the same diet without sulfaguanidine. These workers conclude that this drug inhibits the bacterial synthesis of essential growth factors in the natural vitamin B-complex.

Woolley (*J. Exp. Med.* **75,** *277* (*1942*)) has shown that inositol can be synthesized, under certain conditions, by intestinal flora in mice, and Nielsen, Shull, and Peterson (*J. Nutrition* **24,** *523* (*1942*)) have shown that bacterial synthesis of biotin occurs in the intestine of the rat. Factors affecting the bacterial synthesis of riboflavin, pyridoxine, and the "filtrate factor" have been described by Morgan, Cook, and Davison (*J. Nutrition* **15,** *27* (*1938*)). Lactose favored riboflavin and pyridoxine production, while corn starch favored the produc-

tion of the "filtrate factor." Sucrose had practically no effect on vitamin synthesis.

Taking advantage of recently developed microbiologic technics of vitamin assay, Mitchell and Isbell (*Univ. Texas Publ. no.* **4237,** *125* (*1942*)) have recently described experiments on intestinal synthesis of eight members of the vitamin B-complex. Rats were fed two types of diets. Diet A consisted of ground lean beef, to encourage the production of coliform flora, and diet B consisted of ground lean beef (75 per cent) and lactose (25 per cent), to encourage the development of acidophylic organisms. Some animals on both diets were used in studies of urinary excretion of vitamins. Others were killed in order to obtain samples of the contents of the stomach, upper and lower halves of the small intestine, the cecum, and the colon. Fecal samples from the two groups of animals were studied also. Diets, cecal contents, and feces were hydrolyzed by enzymatic hydrolysis, using a mixture of papain and takadiastase. Urines were assayed without previous treatment. The assay methods were earlier described in detail by Snell and Strong (*Univ. Texas Publ. no.* **4137,** *11* (*1941*)). These workers conclude that thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, inositol, and folic acid are synthesized by intestinal organisms. Attempts were made to estimate the percentage of the dietary intake of each vitamin that was synthesized by bacteria. They believe that most of the syntheses take place in the cecum. They maintain that diet markedly influences the type of flora and number of organisms. These, in turn, are likely to affect the kind and amount of vitamins synthesized.

Taylor, Pennington, and Thacker (*U. Texas Publ. no.* **4237,** *135* (*1942*)) describe experiments with cecectomized rats. Up to the time of weaning the weight of the cecum ranges from 1 per cent to about 8 per cent of the total weight of the digestive tract. At maturity the proportion of cecum to digestive tract has increased to about 30 to 35 per cent. This may account for the fact that very young rats are more susceptible to certain vitamin deficiencies than older rats which have had time to develop appreciable cecal flora.

These workers confirm previous work which indicates that cecectomized animals are not handicapped if they receive a diet adequate in all essential factors. When the Purina ration, which was deficient in several vitamins of the B-complex, was diluted with sucrose, normal animals remained in good health while animals without ceca were severely affected. With the type of diet used in these experiments no cecal contribution of pyridoxine could be noted. It is of interest to note that the colons of cecectomized animals tended to enlarge. This compensatory hypertrophy of the colon may account for the limited syntheses of folic acid, pyridoxine, pantothenic acid, and thiamine that were observed in cecectomized rats.

It would appear, therefore, that the rat possesses limited ability to synthesize many members of the vitamin B-complex by means of microorganisms. Most of these probably develop in the cecum when dietary conditions are satisfactory for the development of cecal flora. These vitamins are not readily available, however, unless the animal resorts to coprophagy, thus liberating the small amount of vitamin B-factors in the fecal pellets. Further experimentation with cecectomized animals and with sulfa drugs, to discourage development of intestinal flora, should eventually lead to a better understanding of ways and means to control the variables encountered in vitamin assay work with animals and it is hoped that such control may bring about a better understanding of the interrelationships of vitamins.

NOTES

Wartime and the Zoo

McKenny (*J. Am. Vet. Med. Assn.* **102,** *190 (1943)*) states that even zoos have nutritional problems in wartime. Many tropical birds and animals, for example, insist on their daily allotment of bananas. The San Diego Zoological Garden established facilities for freezing and storing 30 tons of fish at one time. Some animals have been affected by the temporary shortage of garden produce on the West Coast.

The zoological gardens of England have had to reduce their number of birds and animals owing to lack of food supplies. There is apparently little practical danger of animals escaping, even in highly bombed areas. McKenney states that at the London Zoo, which has been bombed seventy-five times, only "a few harmless zebras" have escaped.

Attendance at English zoos has actually increased since the outbreak of the war, and the value of this form of recreation in maintaining morale has been pointed out. A plea has been made to permit zoological gardens to "carry on," with the contention that they make a real contribution without seriously hindering the attainment of our economic and military objectives.

The Modern Origin of Kitchen Gardens

Our Saxon ancestors certainly had some sort of cabbage, because they call the month of February "sprout-cale;" but long after their days there was little interest in the cultivation of gardens. Religious men, having leisure, and keeping up a constant correspondence with Italy, were the first people among us who had gardens and fruit trees in any perfection, within the walls of their abbeys, priories, and monasteries, where the lamp of knowledge continued to burn, however dimly. In them men of business were formed for the state; the art of writing was cultivated by the monks; they were the only proficients in mechanics, gardening, and architecture. The barons neglected every pursuit that did not lead to war or tend to the pleasure of the chase.

It was not until gentlemen took up the study of horticulture themselves that the knowledge of gardening made such swift advances. Lord Cobham, Lord Ila, and Mr. Waller of Beaconsfield were some of the first people of rank who promoted the elegant science of ornamenting the kitchen quarters and fruit walls.

A remark made by the excellent Mr. Ray in his "Tour of Europe" at once surprises us and corroborates the above statements, for we find him observing that "the Italians use several herbs for sallets, which are not yet or have not been but lately used in England, selleri (celery), which is nothing else but the sweet smallage; the young shoots whereof, with a little of the head of the root cut off, they eat raw with oil and pepper." And further he adds, "curled endive blanched is much used beyond seas; and, for a raw sallet, seemed to excel lettuce itself." Now this journey was undertaken no longer ago than in the year 1663. (Gilbert White, "The Natural History of Selborne.")

Important Foods for British Infants

Since the first of the year 1942 all British infants have received regular allowances of milk, cod liver oil, and orange juice. When parents are unable to afford it, the materials are provided free. As a result, nutrition experts such as Sir John Orr confidently believe that on the average the nutritional status of many British youngsters will be improved under wartime conditions. A recent item in the Lancet (*Lancet* **II,** *460 (1942)*) provides some interesting comments by a subcommittee of the British Pediatric Association. The cod liver oil preparation now supplied by the British government for children under 5 years of age contains not less than 100 I.U. of vitamin D per gram. The dosage recommended is half a teaspoonful for babies under 6 months of age and a

teaspoonful later, thus providing from 200 to 400 I.U. of vitamin D daily. The Committee has recommended that the oil for distribution to children be reinforced with irradiated ergosterol so that a teaspoonful will provide 700 I.U. of vitamin D, in addition to the present content of 3500 I.U. of vitamin A. They suggest that mothers be instructed to give their babies a few drops of this oil daily when the infant is a month old and to increase the amount gradually to a teaspoonful daily within two weeks. For babies born prematurely the committee recommends the administration of 2 teaspoonfuls of the oil, divided into four equal doses and continued during the summer as well as the winter months. Normal babies in their opinion need not be given vitamin D in the summer provided they are exposed to sunlight for at least four hours daily. The subcommittee has also recommended giving 700 I.U. of vitamin D daily to older children on a mixed diet, dropping to 500 I.U. at school age. Pregnant and nursing mothers should receive a supplement of 700 I.U. of vitamin D daily; it is emphasized that this does not take the place of a supplement for the breast-fed infant.

Food Habits

It is always best for intelligent people not to fool themselves, and we might as well admit frankly that it is not an easy matter to change our own food habits, comments Dr. Mark Graubard, Industrial Workers Nutrition Specialist in the Office of Defense Health and Welfare Services, Federal Security Agency. All people are slaves to habit. Knowledge of good nutrition alone does not make people give up bad eating habits.

Our dietary habits and tastes were not selected consciously by any of us but, so to speak, crept up on us in the course of our growth and development. It is not likely that many of our tastes were guided by knowledge of the role which food and vitamins play in the physical and mental well-being of the individual. On the contrary, our eating habits are largely the results of custom and tradition, of the kind of cooking to which our mothers were accustomed, mixed with some personal likes and dislikes. Only recently, with the increased emphasis on better nutrition, has modern nutrition begun to play a role in our dietary habits.

We should enjoy our food but we must not let our personal tastes undermine our health. On the contrary, food should build it up. And this can easily be done by every one of us if we have the will to put it into practice. To achieve this aim it is only necessary that we learn what a good nutritious daily diet consists of, and that we modify our food habits, if necessary, to follow the newly learned principles of nutrition.

"Watch Your ABC's"

Dr. R. J. Williams (*Science* **95,** *340 (1942)*) reviews the known and possible effects of malnutrition upon the body and mental health of both experimental animals and man. On this basis he draws a picture of what might be expected to ensue when mankind can be assured an adequate intake of all the essential food factors. He states: "It should be pointed out that good diets, which mean an abundant supply of vitamins, among other things, promote intellectual keenness as measured by psychologic tests both on animals and human beings. There can be no doubt that much dullness on the part of schoolchildren, particularly among the lower income groups, can be traced in part to a lack of the proper kind of food and specifically to the lack of enough vitamins."